Original Article

Quantification of mast cells in nonreactive and reactive lesions of gingiva: A comparative study using toluidine blue and immunohistochemical marker mast cell tryptase

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Abstract

Background: Mast cells (MCs) are immune cells derived from a multipotent CD34 precursor. The most significant identifying feature of MCs is the presence of metachromatic granules. MCs are increased in oral reactive lesions and are possibly involved in pathogenesis of these lesions.

Objectives:

1. To compare the number of MCs between reactive and nonreactive lesions of gingiva using toluidine blue (TB) and mast cell tryptase (MCT) as a specific marker for MCs 2. To compare the staining specificity/efficacy of TB and MCT.

Methodology: The study sample comprised 90 tissues which were divided into three groups: Group A comprised 30 cases of pyogenic granuloma (PG), Group B consisted of 30 cases of gingival hyperplasia (GH) and Group C comprised 30 cases of pericoronitis. Staining was done between 1% TB and immunohistochemistry (IHC) marker MCT.

Results: A significant increase in number of MCs was observed in PG as compared to GH and pericoronitis. IHC marker MCT proved to be a more specific marker for MCs compared to TB.

Conclusion: IHC marker MCT is a specific marker compared to TB. The position of MCs changed from juxtaepithelial in GH to deeper connective tissue in PG which was in correlation with the proliferating tissue that is epithelium in GH and blood vessel in PG.

Keywords: Mast cell tryptase, mast cells, toluidine blue

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INTRODUCTION

Oral mucosa is constantly subjected to external and internal stimuli which manifest in the form of a spectrum of disease that vary from developmental, reactive and inflammatory to neoplastic.[1] Most common reasons for growths of the gingival tissues are underlying systemic diseases, drug-induced stimulus, local iatrogenic factors or dental plaque.[2]

Most often reactive lesions, which simulate nonneoplastic nodular swelling, develop as a result of chronic and

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Table 1: Comparison of mast cell count stained by two different techniques in pyogenic granuloma

Staining technique	*n*	Mean±SD	*P*
TB	30	52.86±55.74	<0.0001*
IHC	30	192.70±94.08	

*$P<0.05$, statistically significant difference, Mann–Whitney *U*-test. SD: Standrad deviation, TB: Toluidine blue, IHC: Immunohistochemistry

Table 2: Comparison of mast cells count stained by immunohistochemical marker in three different lesions

Lesions	*n*	Mean±SD	*P*
PG	30	192.70±94.08	0.005*,#
GH	30	142.43±58.79	
Pericoronitis	30	122.40±58.10	

*$P<0.05$, statistically significant difference, Kruskal–Wallis test, #*Post hoc* analysis: Significant difference of PG with GH and pericoronitis. SD: Standard deviation, PG: Pyogenic granuloma, GH: Gingival hyperplasia

Table 3: Comparison of mast cells in juxtaepithelial and connective tissue stained by Immunohistochemical marker in three different lesions

Location	Mean±SD			*P*
	PG	GH	Pericoronitis	
Juxta epithelium	99.43±51.49	77.93±34.58	59.30±35.28	0.003*,[a]
Connective tissue	93.26±47.82	64.50±35.07	63.10±29.15	0.005*,[b]

*$P<0.05$, statistically significant difference, Kruskal–Wallis test, [a]*Post hoc* analysis showed significant difference of PG with pericoronitis, [b]*Post hoc* analysis showed significant difference of PG with GH and pericoronitis. SD: Standard deviation, PG: Pyogenic granuloma, GH: Gingival hyperplasia

Table 4: Comparison of mast cell count stained by two different techniques in pericoronitis

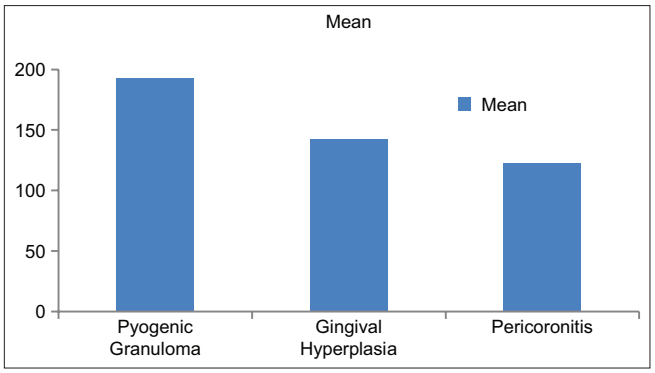
Staining technique	*n*	Mean±SD	*P*
TB	30	51.60±32.57	<0.0001*
IHC	30	122.40±58.10	

*$P<0.05$, statistically significant difference, Mann–Whitney *U*-test. SD: Standard deviation, TB: Toluidine blue, IHC: Immunohistochemistry

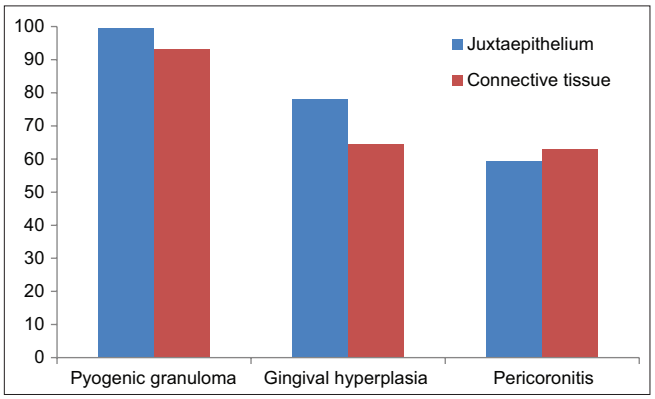
Table 5: Comparison of mast cell count stained by two different techniques in gingival hyperplasia

Staining technique	*n*	Mean±SD	*P*
TB	30	55.80±41.95	<0.0001*
IHC	30	142.43±58.79	

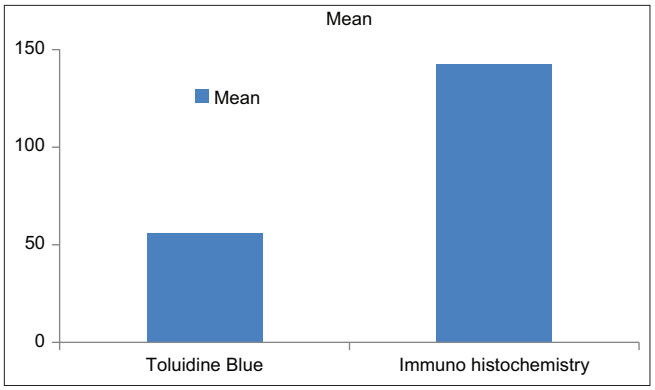
*$P<0.05$, statistically significant difference, Mann–Whitney *U*-test. SD: Standard deviation, TB: Toluidine blue, IHC: Immunohistochemistry



Graph 1: Comparison of mast cells count stained by immunohistochemical marker in three different lesions



Graph 2: Comparison of mast cells in juxtaepithelial and connective tissue stained by Immunohistochemical marker in three different lesions

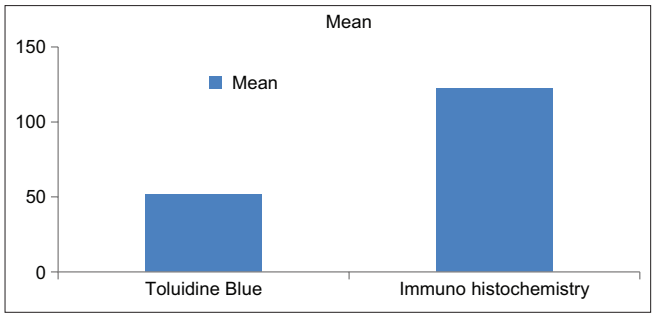


Graph 3: Comparison of mast cell count stained by two different techniques in gingival hyperplasia

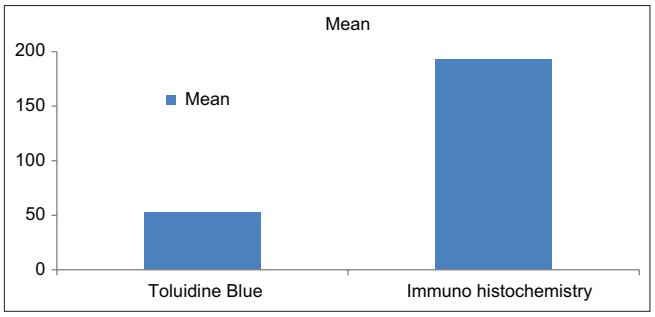
recurring tissue injury which lead to excessive tissue response. These lesions develop commonly in gingiva and present histologically as granuloma gravidarum, fibrous epulis, giant cell epulis, fibroepithelial polyp, peripheral fibroma with calcification, giant cell fibroma or pregnancy epulis.[1]

Mast cells (MCs) are large mononuclear cells. They have a critical role in the development of inflammation in early vasoinductive events and in the transition from acute to chronic inflammation. Hence, MCs are considered crucial in the recruitment of inflammatory cells and angiogenesis.[3,4]

MCs stain with basic dyes such as toluidine blue (TB) and methylene blue.[5] The identification of MC is done by identification of enzymes specific to MCs, mainly tryptase and chymase which is performed through immunohistochemical reaction. Tryptase is found in all human MCs.[5,6] TB is used as a vital stain for mucosal lesions and stains connective tissue mucins, MC granules,



Graph 4: Comparison of mast cell count stained by two different techniques in pericoronitis



Graph 5: Comparison of mast cell count stained by two different techniques in pyogenic granuloma

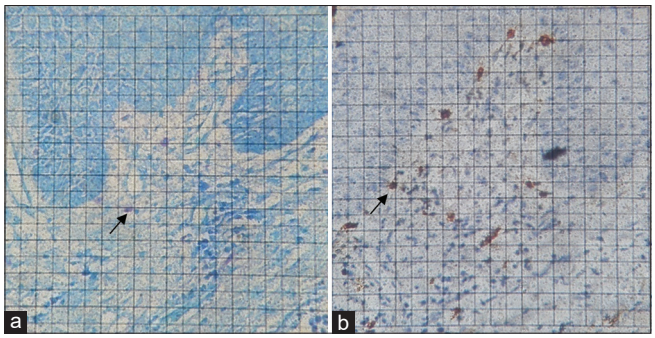
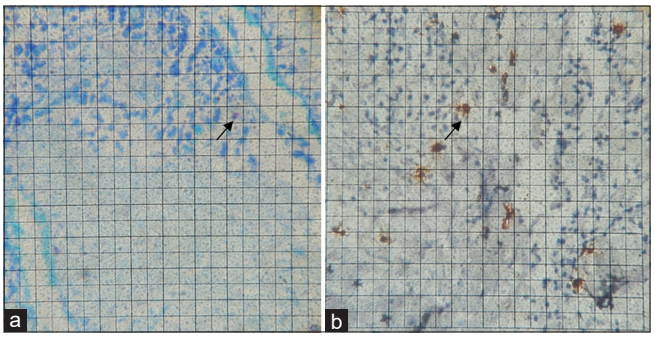


Figure 1: Pyogenic granuloma (juxtaepithelially). (a) Toluidine blue. (b) Mast cell tryptase



7 2: Pyogenic granuloma (connective tissue). (a) Toluidine blue. (b) Mast cell tryptase

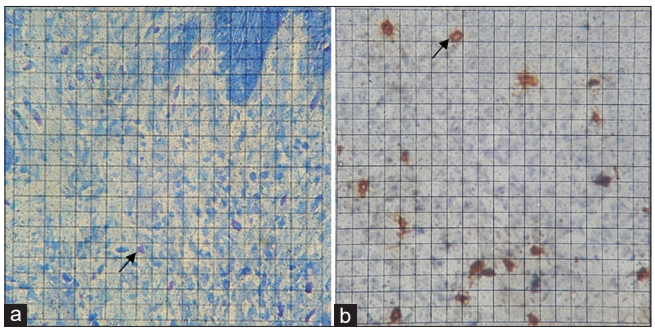


Figure 3: Gingival hyperplasia (juxtaepithelially). (a) Toluidine blue. (b) Mast cell tryptase

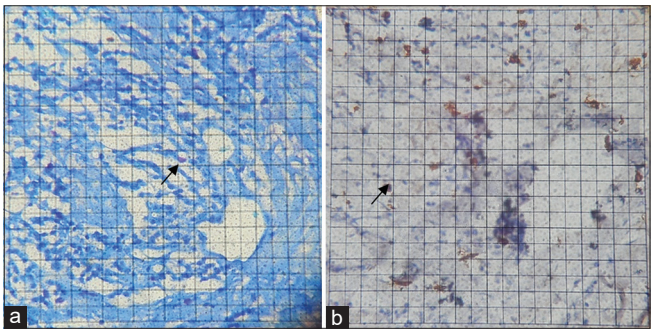


Figure 4: Gingival hyperplasia (connective tissue). (a) Toluidine blue. (b) Mast cell tryptase

amyloid, endocrine cell granules, sulfatides, *Corynebacterium diphtheriae* containing granules with polymerized inorganic polyphosphate, Helicobacterand frozen section.[7] Pyogenic granuloma (PG), fibrous hyperplasia, gingival enlargement and pericoronitis are lesions in which inflammation is seen to a variable extent.

This study aims to compare the staining efficacy of TB with immunohistochemical marker mast cell tryptase (MCT) – for identification of MCs and to quantify them in oral reactive lesions and nonreactive lesions of gingiva.

METHODOLOGY

This case–control study was conducted in the Department of Oral Pathology and Microbiology of Sri Aurobindo College of Dentistry, Indore, and Sharad Pawar Dental College, DMIMS, Wardha, India. The study was approved by the institutional ethical committee. The study sample comprised 90 tissues which were divided into three groups: Group A comprised 30 cases of PG, Group B consisted of 30 cases of gingival hyperplasia (GH) and Group C comprised 30 cases of pericoronitis which was obtained from oral surgery department and periodontics department during routine surgical procedures.

Three serial sections of each tissue specimen were stained separately with H and E stain and 1% TB and immunohistochemistry (IHC) marker MCT (DAKO). Several studies have reported increase in mast cell count in reactive lesions like peripheral giant cell granuloma, GH,

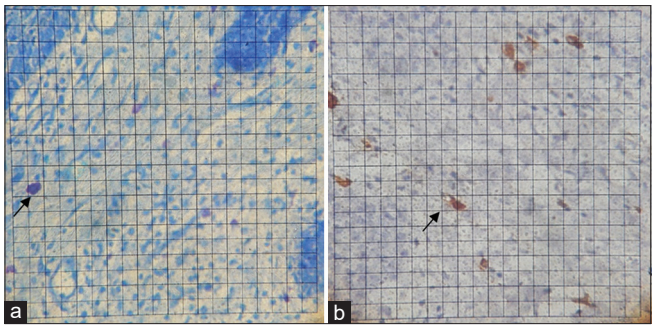


Figure 5: Pericoronitis (juxtaepithelially). (a) Toluidine blue. (b) Mast cell tryptase

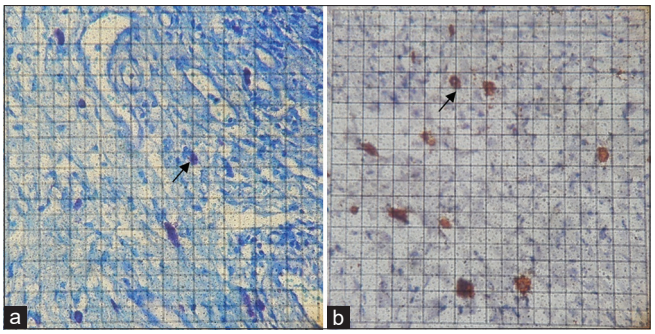


Figure 6: Pericoronitis (connective tissue). (a) Toluidine blue. (b) Mast cell tryptase

Peripheral ossifying fibroma and PG. The areas with tissue folds, calcifications or other processing artifacts were not considered for counting.

Statistical software, Statistical Package for Social Sciences, 20.0 version, IBM, Chicago, was used for statistical analysis. Prevalence of an outcome variable along with 95% confidence limits was calculated. Inferential statistics were used to study the stained MCs per high-power field in normal oral mucosa, PG and GH between TB and MCT.

Kruskal–Wallis test is performed which is a nonparametric equivalent to ANOVA. Here, data showed nonparametric distribution; hence, Kruskal–Wallis test is performed. Whenever a test is performed and if there is a significant difference, then *post hoc* analysis is performed to know that between which two groups significant difference exists.

OBSERVATIONS AND RESULTS

A total of 90 subjects equally distributed in three groups pericoronitis, PG and GH, were randomly selected for the study. For test group, all individuals in the age range of 20–70 years having reactive lesions on gingiva were selected and random selection of male and female. For control group, healthy individuals with nonreactive lesion of gingiva (pericoronal flap tissue) within the age range of 20–70 years were selected.

DISCUSSION

MCs are immune cells derived from a multipotent CD34 precursor and metachromatic cytoplasmic granules are the characteristic features of MCs.[4]

Several studies have reported increase in mast cell count in reactive lesions like peripheral giant cell granuloma, GH, Peripheral ossifying fibroma and PG.[8,9]

In our study, we included PG and GH among all reactive lesions and pericoronitis among nonreactive lesions because they have common predisposing factors such as plaque, local debris and microorganisms; hence, we felt by including these lesions the baseline parameter of etiology would become common and thus help to understand the role of MCs better.

Previous literature has shown that in inflammation, MCs play a important role,[10] which incidently is also one of the features of GH, pericoronitis and PG.

H and E is the routine stain used in laboratories for diagnosis of reactive lesions, but MCs are difficult to visualize by H and E stain, as they share the same staining characteristics as the fibroblasts and hence are difficult to differentiate from them.

MC granules stains purplish red and nuclei sky blue in color with a metachromatic dye, TB, which makes them easily identifiable against a blue background.[11] The advantage of TB stain is its low cost and ready availability.

Tryptase is one of the main components of MC which is an enzyme found exclusively in these cells. MC granules are round, oval, spindled or stellate shaped and they stained reddish brown in color by tryptase.[12] MCT facilitates clear, accurate and rapid count. Tryptase is considered as a specific MC marker based on reaction restricted entirely to the granules of MC.

de Oliveira Rodini *et al*.[13] mentioned that the long-standing procedure for MC identification for heparin based on metachromatic staining is less reliable, as the technique may stain cells such as macrophages and fibroblasts because of free mastocyte granules from bodily function and it should fail to stain immature MCs.

Therefore, to avoid this in our study, we use H and E for confirmation of the lesions and TB along with immunohistochemical marker (IHC) MCT as a special

stain and specific marker for MCs. Thus, we aimed to have a direct comparison between TB and IHC markers for identification of MCs.

Several studies have been done on MCC in PG, drug-induced GH and other reactive lesions but not in inflammatory GH. As far as our knowledge goes, our study is the first one in which PG, inflammatory GH and pericoronitis have been correlated with respect to the quantification of MCs.

Sheethal *et al*.[9] conducted a study and they found that the mean value of MCC in granuloma gravidarum was 79.54 ± 70.51 per high-power field. They used TB stain for counting of MCs.

In our study, the mean value of MCs in PG by IHC was 192.70 ± 94.08 and by TB stain, it was 52.86 ± 55.73 [Table 1 and Figures 1,2].

On comparing the number of MCs in reactive lesions of gingiva, we found that MCC to be maximum in PG followed by GH and pericoronitis [Table 2].

In support of our study, Kamal *et al*.,[14] found that MC number was increased in granuloma gravidarum as compared to normal oral mucosa when stained with 1% TB.

In the present study, on using TB, no significant difference was observed in the MC count among three lesions. However, on using MCT the MCC was found to differ significantly between the lesions [Graph 1]. This indicated greater efficacy of MCT over TB in detection of mast cells. The difference in the efficacy of two techniques can be attributed to the fact that the immunohistochemical staining utilizes the principle of antigen antibody reaction (Sturdy membrane) and thus identifies both granulated as well as degranulated mast cells, whereas, TB utilizes the principle of metachromatic staining and thus can not detect the degranulated mast cells leading to lesser mast cell count.[15]

Literature stated that MCs were found in different areas of periapical and gingival lesions, distributed in several patterns: subepithelially, in isolated groups in connective tissue and as isolated cells. Some cells were seen in association with blood vessels, some in association with fibroblasts and several in association with lymphocytes.[9]

We tried to simplify this by dividing the connective tissue zone in these lesions as juxtaepithelial and deeper connective tissue areas in order to determine if the location of MCs had any correlation with the type of lesion. We found that juxtaepithelially MCs were more in GH, followed by pericoronitis and PG indicating a probable role of MC in epithelial proliferation in GH [Table 3 and Graph 2].

In our study, connective tissue MCs were found to be increased in PG followed by GH and pericoronitis, indicating their probable role in vascularization. PG clinically tends to bleed as it is highly vascular.[1]

Kamal *et al*.[14] conducted a similar study in which they compared MCC in normal oral mucosa with granuloma gravidarum and tried to correlate MC function in pathogenesis of PG. They concluded that MCC is higher in oral granuloma gravidarum, suggesting its role in the recruitment of inflammatory cells and angiogenesis.

In pericoronitis the MCC was less than that in PG and GH in both juxtaepithelial and connective tissue areas. This probably could be due to pericoronitis is being transient inflammatory lesion and not an established lesion as PG or GH as pericoronitis will regress on its own contrasting to PG and GH which need to be excised or surgically treated. In pericoronitis, there is epithelial proliferation which is in response to the inflammation and associated vasodilation which decreases when conditions improve, so the MC distribution was generalized and also the count was less compared to PG and GH [Tables 1,4,5 and Graph 3-5, Figures 1-6].

Thus, we proposed that unless a cause is known and identified chronic localized inflammatory GH could actually be a healing or more precisely fibrosing PG.

CONCLUSION

We can say that MCCs are more in PG followed by GH and pericoronitis. IHC marker MCT is definitely a better option over TB due to its crisp staining. However, where cost is an important factor there, TB can be used as a cheaper alternative. We can also postulate that as chronic localized GH could actually be a fibrous counterpart of PG; therefore, the position of MCs are juxtaepithelially in fibrous hyperplasia and pericoronitis, while they are more in deeper connective tissue in close proximity to blood vessels in PG.

Further, the scope of the study lies in microbial culture to determine type of causative microbes in PG, GH and pericoronitis to see if any difference exists at the bacterial level.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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